Project title:	**HNS: A study of the benefits of mycorrhizas in containerised production systems.**
Project number:	**HNS 99**
Project leader:	**Dr Jim Monaghan** **HRI-Wellesbourne** **Warwick** **CV35 9EF**
Report:	**Final report (December 31st 2001)**
Previous reports:	**Annual report 2000**
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Date commenced:	**1st June 1999**
Key words:	**HNS, container grown, mycorrhizas, propagation**

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Practical Section For Growers

Background and Objectives

Mycorrhizas (symbioses between fungi and roots) have evolved to confer benefits on both partners. One kind in particular, the AMF, has apparently a great potential for use in reducing or replacing the application of inorganic fertilisers and pesticides in HNS plants. Scientific research has shown repeatedly that, in carefully controlled laboratory and greenhouse conditions, plants with a suitable mycorrhizal fungus out-perform those lacking such a symbiosis. However, it has consistently proved very difficult to replicate such results in the 'real world' of nurseries, gardens and forests.

Recent developments in mass production of AMF have resulted in several products being marketed for use in such situations as HNS production. The conditions used in glasshouses and nurseries should be ideal for reaping the putative benefits of mycorrhizas. Usually, substrates are free of native AMF species, and conditions of water supply, lighting and plant care are optimised. However, there are two factors in HNS production that might militate against successful and profitable application of mycorrhiza technology. Firstly, high rates of inorganic fertiliser can eliminate or reduce root colonisation by AMF. Secondly, use of 'biologically active' peat substrates may suppress root colonisation.

The overall aim of the project was to determine whether the incorporation of commercial AMF products could **increase the profitability of containerised HNS**, through a reduction in fertiliser and pesticide inputs. This aim was pursued by answering the following questions (each being an experimental objective):

1. Are the **AMF in commercial products viable** (and are their component fungi correctly identified)?

2. Do AMF **improve rooting success** if used in a peat-based propagation mix?

3. Can plants **use less CRF** if AMF are included in a peat-based growing mix?

4. If so, is this through increased **nutrient use efficiency**?

5. Do AMF protect plants from **root pathogens**?

The experimental work was divided into three components: identification of AMF in commercial products undertaken by Dr Chris Walker (BRIL); plant growth studies led by Dr Jim Monaghan (HRI-Wellesbourne); and pathology study carried out by Dr Tim Pettitt (HRI-Wellesbourne). All plant experiments were carried out at HRI-Efford from 1999-2001.

Summary of Results

Bioassay (Years 1 & 2)

Three AMF containing products were studied: PHC Nursery Media Mix; Symbio MycoForce Potting Mix (Endo) and Vaminoc.

Three batches of product were obtained over time and examined.

1. The examination of commercial products showed that AMF fungi were present, though not always in large quantities (judging from spore numbers). Spores were probably the main viable propagules in arbuscular mycorrhizal fungi, especially when their substrate had undergone desiccation. The species present did not, for any inoculum, coincide completely with those said to be included. This should be a cause for concern, as it may be indicative of a need for much better quality control during production, and checking before marketing.

2. Of the species present as spores, not all successfully sporulated. This could be because the spores were initially dead, or the fungi did not compete successfully with others in the mixture, or because the substrates or hosts were inappropriate for the particular fungi. Further work is necessary to establish the underlying cause of these responses.

3. There were indications from this work that some factor in peat is limiting AMF colonisation - whether this is due to organic content or pH could not be established from this work.

Propagation study (Year 1)

This work studied the effect of including AMF products in the rooting media of four species (*Magnolia x soulangeana* 'Rustica Rubra'; *Choisya ternata*; *Lavandula angustifolia* 'Hidcote' and *Chamaecyparis lawsoniana* 'Columnaris').

1. Some improvements in cutting establishment were observed with the addition of the commercially prepared AMF products. However the **results were generally disappointing** from the point of view of the use of AMF products to improve the propagation of cuttings of HNS. Only *Magnolia* x *soulangeana* with one product (Vaminoc) resulted in consistent colonisation and mycorrhiza production, but this failed to produce a significant benefit.

2. In some instances, a **factor or factors within these products not attributed to the mycorrhizal fungi appeared to be associated with improvements in cutting survival** and subsequent growth. This was most marked in *Lavandula* where rooting success was significantly improved (Symbio Mycoforce Potting Mix (Endo), Vaminoc - autoclaved and 'live') and the rate of rooting increased (Symbio Mycoforce Potting Mix (Endo), Symbio Mycoforce Potting Mix (minus AMF propagules) and Vaminoc - autoclaved and 'live'). These responses were not associated with AMF colonisation of the roots.

3. Some treatments were associated with reductions in rooting success but in this study it was not possible to unequivocally establish the cause(s) of these plant losses.

Growing-on study (Year 2)

This work studied the effect of including the three commercial AMF products studied in Year 1 and three isolates of known provenance (*Paraglomus occultum, Glomus sp. and Archaeospora gerdemannii)* in the growing media of three species (*Chamaecyparis lawsoniana* 'Columnaris', *Choisya ternata*, *Lavandula angustifolia* 'Hidcote').

1. ***Chamaecyparis*** showed **good root colonisation** following incorporation of AMF propagules in the growing mix. Growth responses in general were correlated with presence or absence of AMF. Interestingly, nutrient level influenced plant response to colonisation: at high nutrients plant growth was reduced; at low levels of nutrient growth was increased.

2. Colonisation of *Chamaecyparis* roots with *Paraglomus occultum* **enhanced growth at the low nutrient level** but not so much as to substitute for a higher CRF rate. At the higher nutrient rate this effect was reversed. Symbio Mycoforce Potting Mix (Endo), gave marked growth promotion with the 'no propagules' carrier mix at the high nutrient rate. However, root colonisation was very high suggesting that this treatment contained viable propagules.

3. ***Choisya*** showed **low root colonisation** following incorporation of AMF propagules in the growing mix. In contrast to *Chamaecyparis* plant growth responses was not associated with presence or absence of AMF. The lack of colonisation explains the lack of response to treatment. However, a significant effect was observed with *Glomus sp.* and *Archaeospora gerdemannii* where some **unexplained factor** was responsible for marked growth

4. ***Lavandula*** showed **general colonisation** across all treatments. The level of colonisation was generally greater in the treatments inoculated with viable propagules. It is likely that this contamination was introduced during the commercial propagation of the plugs bought in for the study. Plant **growth did not respond to treatment** or extent of colonisation of roots

except for autoclaved PHC Nursery Media Mix, which suppressed growth significantly at the high rate of CRF. This last response is unexplained.

5. Over all three HNS species, nutrient uptake was influenced by AMF colonisation, with the general relationship showing that to some extent **high AMF colonisation was associated with low concentration and weight of Mg and P in the foliage**. In contrast to the general trend, greater colonisation of roots by PHC Nursery Media Mix treatments was associated with increased concentration and weight of P in the foliage.

Pathology study (Year 2)

This work involved two experiments studying the incidence and severity of *phytophthora* root rot disease in *Chamaecyparis lawsoniana* 'Columnaris' colonised by three AMF treatments (chosen as they had previously been shown to colonise well):Vaminoc; *Paraglomus occultum* and *Glomus* sp.

1. Along with the expected foliar symptoms of *Phytophthora* root rot (foliage greying, followed by wilting shoots and plant death), a **shoot 'tip-hooking' symptom** was observed in both experiments 1 and 2 and the incidence of this symptom was strongly associated with root browning.

2. **Negligible root browning** was recorded in the treatment incorporating **'live' *Paraglomus occultum***, and also comparatively reduced root browning was seen in the other two 'live' AMF treatments. Surprisingly, the no AMF controls in experiment 1 showed a comparatively low incidence of infection and consequently of root browning.

3. **No symptoms of *Phytophthora* root rot** were seen in any of the live' ***Paraglomus occultum*** **treated plants** Other treatments all showed some foliar symptoms of disease, although symptom severity at the time of recording the experiments was still low.

4. ***Paraglomus occultum*** **gave good disease control in both experiments**. This could be related to the high level of root colonisation achieved with this AMNF species and it shows great promise for disease control in the future.

Action Points

- As AMF propagules in these products are grown with living plants **there is the possibility that pathogens could inadvertently be included**. Clearly it is important to eliminate any such possibility through high levels of quality control. The inclusion of plant pathogens in commercial products would not only be to the detriment of inoculated plants, but would be in breach of plant health regulations, particularly in those products imported from outside the European Union.

- **The results of this work do not support the general application of commercial AMF to a peat based propagation or growing mixes**

- **No benefits in commercial rooting success were gained from the inclusion of AMF**. Interestingly, factors other than AMF significantly improved rooting survival and cutting growth. If these positive results with 'beneficial' components were repeatable this could be an area of financial benefit for propagators of young plants.

- Under the conditions of this study growth increases in a 3 litre containerised plant were rarely associated with AMF colonisation, and where observed, were **not large enough to overcome the loss in plant growth due to the necessarily reduced CRF rate**.

- If suitable isolates can be produced commercially AMF use **may reduce root rot diseases** and lead to a **reduction in fungicide applications**.

These studies have highlighted three avenues worthy of further research:

a) The potential for **AMF to reduce plant losses** (and reduced quality) due to root disease

b) The isolation and testing of AMF from environments similar to those in which they are to be used commercially i.e. from a peat substrate. This is probably the reason that *Paraglomus occultum* was the most successful treatment.

c) The obvious growth responses to the other '**beneficial biologicals'** included in some of the commercial products studied, often quite marked growth increases.

Science Section

Introduction

The term mycorrhiza refers to an intimate association between plant roots and certain soil fungi. There are five types of mycorrhiza, but those of chief interest to containerised hardy nursery stock (HNS) production are ectomycorrhizal fungi and arbuscular mycorrhizal fungi (AMF). Ectomycorrhizal fungi form associations with about 3% of higher plants, mainly forest trees in the Fagaceae, Betulaceae, Pineacea, *Eucalyptus* and some woody legumes. In contrast, AMF form associations with members of about four-fifths of all land plant families, including the vast majority of HNS species. Notable exceptions are the Ericaceae which have their own types of mycorrhiza, and a few non-mycorrhizal plant families, e.g. Caryophyllaceae and Brassicaceae.

Much of the research carried out in the last 30 years suggests that AMF confer advantages on their host plants in several ways (though not necessarily all in the same plant-fungus-substrate combinations). These include enhanced nutrient transfer, particularly phosphorus, ammonium, zinc and copper (Barea, Azcon & Azcon-Aguilar, 1993); improved rooting of cuttings (Chang, 1994); increased protection against root pests such as some nematodes (Carling, Roncadori & Hussey 1996) and pathogenic fungi, eg. *Phytophthora, Gaeumannomyces, Fusarium, Thielaviopsis, Pythium, Rhizoctonia, Sclerotinia, Verticillium, Aphanomyces* (reviewed by Linderman, 1994 and Hooker *et al.,* 1994). In addition, there is some evidence suggesting that AMF may be associated with increased drought tolerance, probably through associated changes in the structure of the growing media (Barea *et al.,* 1993).

Plants grown in substrates inoculated with suitable AMF require lower levels of nutrient input and grow better than non-mycorrhizal plants (Bolan, 1991). Containerised production systems such as those in the ornamental plant industry are ideal for effective use of AMF because they use defined, disinfested media (without unwanted AMF) that can be manipulated to incorporate the most beneficial AMF for a relatively short (1 – 2 year) growth cycle. These factors increase the likelihood that mycorrhizas will contribute significantly to profitable plant production in such systems. In the USA, where commercial inocula are available from about 16 companies, the use of mycorrhizas in production systems is apparently increasing in response to the environmental lobby, and it is anticipated that the same pressures will come to bear on UK growers in the future.

Over the last few years commercially produced mycorrhizal inocula have been introduced to the UK market by a number of companies. These products contain either AMF spores alone or a mixture of spores and small root fragments containing living AMF propagules considered to be capable of colonising new host root tissue. All the products are described as containing a number of species of AMF.

Although AMF have demonstrated potential for use in the containerised HNS sector, a number of disadvantages must be considered. AMF will not normally confer benefit (indeed, may not colonise roots) in a high nutrient environment; some of the 'younger', less mature blonde peats may limit colonisation of roots by certain species of AMF; some pesticides may detrimentally affect AMF (Azcon-Aguilar & Barea, 1997). Companies producing commercial preparations of AMF have carried out screening programmes for their products and this information is available from their respective sales personnel.

The overall aim of this project is to determine whether the incorporation of current AMF products increases the cost-efficiency of containerised HNS, through a reduction in fertiliser and pesticide inputs. Environmental issues will be addressed through potential to reduce both inputs and nutrient leaching.

The following objectives constitute the components of the overall experimental programme. The entire team are closely involved in all aspects, but the principal worker (or workers) on each objective is identified:

This final report covers in depth objectives 1, 3, 4 and 5, and summarises objective 2.

1. **To identify the AM fungi in the commercial products, and to test their viability by checking their presence before and after a period of plant growth (quality control) -- C Walker.**

2. **To determine whether addition of AMF improves rooting success of cuttings[1] -- J Monaghan.**

3. **To determine whether addition of AMF will allow rate of CRF application to be halved without loss of production quality -- J Monaghan.**

4. **To determine whether AMF inoculation of plants leads to a more efficient accumulation of nutrients -- J Monaghan.**

5. **To determine the impact of AMF colonisation on plants' susceptibility to root rot pathogens - T Pettitt.**

[1] Initially it was intended to compare the performance of plants inoculated at rooting and potting on, but plant losses at the propagation stage rendered this impossible – see results and discussion.

Analysis and bioassay of commercial products (Year 2)

Bioassay 1 & 2 are described in the previous report

To identify the AM fungi in the commercial products, and to test their viability by checking their presence before and after a period of plant growth (quality control)

Materials and Methods

The products were obtained directly from the suppliers and sampled. Spores were extracted by a centrifugation-sugar floatation method (Walker *et al.,* 1982) and then washed on a 35 micrometre sieve to remove fine material smaller than any spores, and then examining the entire amount left on the sieve, a spatula-end at a time (suspended in water in a 6-cm Petri). If spores were found, samples of each spore type were preserved in polyvinyl alcohol lacto-glycerol on microscope slides and identified under a compound microscope at magnifications of up to ×2000. Photographs were taken and voucher specimens were preserved in a permanent herbarium with an appropriate sequential number.

Results

Examination of products.

PHC Nursery Media Mix

The PHC inoculum contained four recognisable species of arbuscular mycorrhizal fungi (Plate 1.). *Glomus intraradices* and *Glomus etunicatum* were present in large quantities, These were in the list of species that were given in the product details. A species from a different genus, *Acaulospora longula*, was also recovered in large numbers. This species did not appear on the list of contents, but it is known to be an arbuscular mycorrhizal fungus.

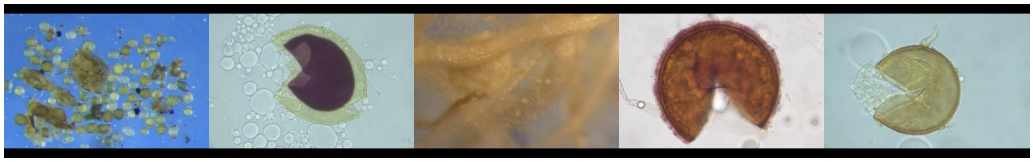


Plate 1. Spores from the PHC Nursery Media Mix treatments.
Left to right: mixed spores as extracted from a sample of the inoculum before use; spore of *Acaulospora longula* stained with Melzer's reagent to give a purple reaction of inner walls); *Glomus intraradices* spores in and around roots; *G. intraradices* single spore showing red reaction of outer wall to Melzer's reagent; *G. etunicatum*.

Symbio MycoForce Potting Mix (Endo)

There were no spores or other recognisable propagules found in first batch. A few spores were recovered from the dried inoculum of the second batch, but they were rather difficult to identify because they were in very poor condition, lacking any evident cytoplasm. The species recovered were identified as *Glomus etunicatum*, *Glomus intraradices* (perhaps – see relevant comments on the species below), and another species, possibly *Glomus claroideum (Plate 2.)*. This last was present only in very low numbers of spores, and they were particularly badly deteriorated. None of the species was recovered from the bioassays. Although mycorrhizas did form in a few of the main experimental plants, these were inconsistent, and were likely to be contamination.

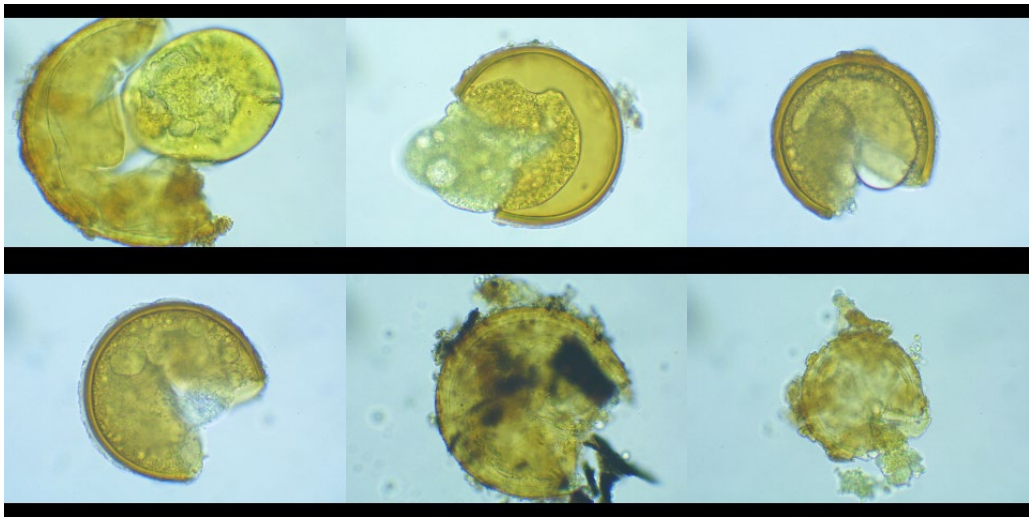


Plate 2. Spores from the Symbio MycoForce Potting Mix (Endo) inoculum.
Top left to right: possible *Glomus intraradices* spore in very poor condition (rendering it impossible to identify with certainty); *Glomus etunicatum* spore with partly congealed spore contents; *Glomus etunicatum* spore in apparently good condtion (with contents as oil globules). Bottom left to right: *Glomus etunicatum* spore with oily contents, probably in good condition; possible *Glomus claroideum* spore in very poor condition with congealed contents; possible *G. claroideum* spore, again in very poor condition.

Vaminoc

The Vaminoc inoculum contained only *Glomus* spp. (Plate 3.). The predominant fungus was *Glomus mosseae*, but others were present in low spore numbers. The second most frequent species was an undescribed fungus, corresponding to one originally isolated from Rothamsted Experimental Station, which has been known as 'E3'. Another, fungus, *Glomus* sp., also corresponded with an early Rothamsted isolation known as 'Red Brown Laminate'. This has never been properly identified, and was present in such low numbers that confirmation of identity was impossible. One or two other species were also present, but again in very low numbers. The two species, *Glomus intraradices* and *Glomus aggregatum*, are somewhat inadequately described in the literature, and may be different morphs of the same fungus. From species descriptions, both these fungi were present in the dried inoculum, but neither these, nor the RBL species were successfully established in the bioassays or main trials. The bioassays all produced *G. mossseae,* and *Glomus* '3'.

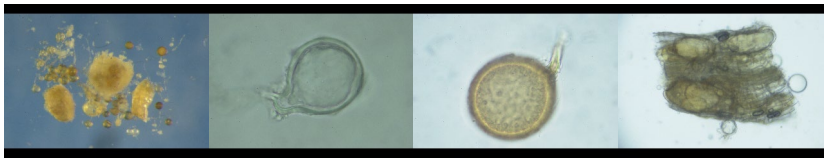


Plate 3. Spores from the Vaminoc treatments.
Left to right: *Glomus mossea* (spores singly or in groups); undescribed *Glomus* 'E3'; *Glomus* 'RBL' (perhaps *Glomus macrocarpum*); *Glomus aggregatum* (in root fragment).

Bioassay 3

Materials and Methds

The bioassay was established studying the three commercial inocula and an untreated control treatment, with two highly mycorrhizal plant species, Chrysanthemum (*Dendranthema grandiflora*) and narrow-leafed plantain (*Plantago lanceolata*). These were planted (three replicates of each treatment) in 1.5 litre pots on the 14 September 2000. Two substrates were studied: peat and sand. Both substrates were heat sterilised for 80 mins at approx 90°C. Ficote 270 was added at 0.7 g per pot prior to the placing the substrate in the pots, and 5 g of each product was added into the planting hole and seedlings or tissue-culture plants were planted above the inoculum. Plants were grown in a greenhouse for 8 months before being destructively assessed for AMF colonisation on 31 May 2001.

The bioassay pots were sampled in two ways. First, AMF propagules were extracted from the substrate. Second, the plants were washed from the substrate, and samples of roots were cleared and stained so as to be able to see any mycorrhiza formation. The above-ground portion of each plant was oven-dried and weighed so that comparisons could be made, within each plant species, of any effects of inoculum application on a major growth parameter.

Results

The only AMF spores were extracted from the pots containing sand substrate that had received the Vaminoc inocula, with both host plant species. No spores were present in any other pots. Root assessment showed that only the Chrysanthemum and *Plantago* plants grown in sand incorporating Vaminoc had been colonised by AMF. However, growth responses were observed with the other treatments.

With *Plantago*, substrate and AMF treatment affected plant survival. All plants grown in sand survived but in peat all control and Vaminoc treatments died before the end of the bioassay, and 50% of plants inoculated with Symbio died. Interestingly, all plants inoculated with PHC survived. When the biomass of *Plantago* was analysed, AMF treatment had no effect on plant growth whereas substrate had a marked effect, with peat producing plants 40% smaller than sand.

Different responses were observed in the Chrysanthemum. All plants survived to the end of the trial but biomass showed clear responses. As with *Plantago* plants grown in sand were significantly larger than those in peat but, in contrast, AMF had a main effect with PHC and Symbio associated with significantly larger plants than either Vaminoc or the untreated control plants.

When the survival rates of *Plantago* and biomass of Chrysanthemum are viewed alongside the spore and root assessments, it would appear that these growth responses are due to other properties of PHC and Symbio products (both of which contain a range of beneficial bacteria and plant extracts; see Appendix 1) and not the AMF allegedly contained.

Bioassay 4

Materials and methods

Two other AMF isolates were used to further study the effect of growing substrate. One of the cultures, corresponding to the description of *Paraglomus occultum*, was isolated from a

commercial potting mixture (Levington Alpine Compost), and it was therefore thought that this might be a good performer in peat-based substrates. The other, an isolate currently being described as a putative new species of *Glomus*, came from a sand dune community in Poland, and thus might have been expected to perform well in sandy substrates.

Three species of host; Mallow (*Lavatera trimestris*) Chrysanthemum and Fuchsia; were grown in a range of substrates varying in their proportions of peat:sand (4:0; 3:1; 2:2; 1:3; and 0:4 giving five substrate treatments). The peat (Shamrock) studied was not sterilised, but the sand was sterilised by autoclaving twice on successive days for 1 hr each time. 100 spores of *P. occultum* and 50 spores of the Polish *Glomus* were selected for each treated pot and washed on to roots of plant *in situ* in the planting hole. Seedlings were washed free of sand, and tissue culture medium was washed from plantlets before planting. Five replicates of each substrate x AMF combination were established for each of the three host species. The treatments were set up 11 July 2001, and plants were grown in a greenhouse for 5 months before roots were sampled as described for Bioassay 3.

Results

No AMF were observed on the roots of any host plant where the proportion of peat in the mix was greater than 25%. Where there was no peat or 25% peat in the substrate (0:4 and 1:3 peat:sand, respectively) AMF were found in the all the roots examined from all three host species inoculated with the *Glomus* sp. Colonisation was more variable for *P. occultum*, and interestingly, colonisation was only observed in the 25% peat mix (1:3). No colonisation was observed in the sand only (0:4) or 50 - 100% peat treatments (2:2, 3:1, 4:0).

Substrate obviously has an important role in AMF colonisation, making the succesful utilisation of AMF in commercial production a difficult prospect unless the host x substrate x AMF combination is correct.

Summary

The initial examination of commercial products showed that AMF fungi were present, though not always in large quantities (judging from spore numbers). Spores are probably the main viable propagules in arbuscular mycorrhizal fungi, especially when their substrate has undergone desiccation. The species present did not, for any inoculum, coincide completely with those said to be included. This should be a cause for concern, as it may be indicative of a need for much better quality control during production, and checking before marketing. Even the species present as spores did not all successfully sporulate (and can therefore be presumed not to have colonised). This could be because the spores were initially dead, or the fungi did not compete

successfully with others in the mixture, or because the substrates or hosts were inappropriate for the particular fungi. Further work is necessary to establish the underlying cause of these responses.

Growing substrate obviously has an important role in AMF colonisation, making the successful utilisation of AMF in commercial production a difficult prospect unless the host x substrate x AMF combination is correct. It appears from the bioassays that the commercial AMF where colonising, do so more consistently in sand. There are indications from this work that some factor in peat is limiting AMF colonisation - whether this is due to organic content or pH could not be established from this work. Additionally the range of isolates studied was small and AMF isolates more suitable for use in peat growing media could certainly be isolated from suitable ecosystems. A few are already identified in scientific collections, but there is a great potential for the discovery, isolation and testing of new cultures that would be likely to have more potential as growth-promoters or in pathogen control in nursery stock production.

Crop physiology study (Year 2)

The propagation study (year 1) is described in the preceding annual report.

To determine whether addition of AMF will allow rate of CRF application to be halved without loss of production quality.

To determine whether AMF inoculation of plants leads to a more efficient accumulation of nutrients.

Materials and Methods

Plants were bought in from commercial propagators as rooted plugs and potted on into 3 litre pots. *Choisya* and *Lavandula* were potted 7 March 2000 and *Chamaecyparis* 8 June 2000. The following mixes were used.

***Choisya ternata* & *Chamaecyparis lawsoniana* 'Columnaris'**	***Lavandula angustifolia* 'Hidcote'**
100% Premium Irish peat	75% v/v Premium Irish peat
750 g m^{-3} suSCon Green	25% v/v Perlite
1.5 kg m^{-3} Magnesian Limestone	750 g m^{-3} suSCon Green
	2.5 kg m^{-3} Magnesian Limestone

Controlled release fertiliser (Osmocote 12-14 Exact Standard 15+9+9+traces) was incorporated, as the source of nutrients, at two rates per species 4.5 & 1.1 kg m^{-3} for *Choisya* & *Chamaecyparis* and 2.5 & 0.6 kg m^{-3} for *Lavandula*.

The following **commercially available products** were studied in the trial (for contents see appendix 1) and were common to the preceding propagation trial:

1. PHC Nursery Media Mix*
2. Symbio MycoForce Potting Mix (Endo)
3. Vaminoc

Each product was included at manufacturer's recommended rates and also in a form where the AMF propagules were either absent or killed to study the effect of the 'carrier material' alone on

the growth of the plants. For Vaminoc, a product containing only AMF, autoclaving was sufficient. Symbio supplied the carrier material alone allowing study of the additional role of the AMF in the product. PHC were unable to separate AMF from the carrier material hence any difference in response between the autoclaved and non-autoclaved treatment may be due to the AMF and/or beneficial soil bacteria and fungi. There will also have been some mineralisation of nutrients as the autoclaved organic material decayed. This must be borne in mind when considering the results. In addition, three **AMF isolates of known provenance** from the collection of C. Walker were studied. The treatments studied are summarised below.

Table 1. AMF treatments incorporated into the growing media and abbreviations used in the results.

Treatment	Abbreviation
No AMF product	No AMF
PHC Nursery Media Mix	PHC+
autoclaved PHC Nursery Media Mix	PHC-
Symbio MycoForce Potting Mix (Endo)	Symbio+
Symbio MycoForce Potting Mix minus AMF propagules	Symbio-
Vaminoc	Vaminoc+
autoclaved Vaminoc	Vaminoc-
Paraglomus occultum (Att 694-1)	AMF 1+
autoclaved *Paraglomus occultum*	AMF 1-
Glomus sp. (Att672-2)	AMF 2+
autoclaved *Glomus sp.*	AMF 2-
Archaeospora gerdemannii (Att 200-9)	AMF 3+
autoclaved *Archaeospora gerdemannii*	AMF 3-

The treatments were incorporated into the growing medium by placing a measured quantity of material beneath the plug at potting. This ensured that as the new roots grew from the plug they would pass through the material, maximising the probability of successful colonisation.

Plants were grown under conditions suited to mycorrhizal colonisation whilst still having relevance to commercial production in the UK. It was important that no fungicides were used in this trial, although the treatment of the plug material before despatch was not known. Plants were grown in well vented mesh sided poly tunnels at HRI-Efford. To prevent water-logging, great care was taken to ensure there was no over-watering. Additionally, pots were placed on upturned saucers to eliminate the chances of contamination from the standing area.

* PHC also recommended that a mix of Bio-Pak (see appendix 1) was watered onto the growing substrate through the trial – for comparison with Symbio's product this was watered on three times only

Plants were laid out in a randomised block design with three replicates per treatment. Each experimental unit consisted of 5 plants. The beds were guarded on all sides with untreated plants.

At the end of the trial plants were qualitatively scored for commercial quality characteristics, and the top growth of 3 plants per plot of 5 was removed for dry weight. Foliage samples were collected for analysis of nutrient content (HRI-Wellesbourne). Roots were sampled from each plot, and cleared and stained by the standard method of heating in KOH to remove alkali-soluble pigments, followed by bleaching, where necessary, and either ink or methyl blue to stain the fungus inside the roots. This enabled a visual assessment of the extent of root colonisation by AMF. Roots were assessed for two criteria: AMF level (defined here as the proportion of root length colonised by mycorrhiza), and AMF density (defined here as the proportion of root cells containing mycorrhizal structures).

Results

Chamaecyparis lawsoniana 'Columnaris'

Colonisation by AMF

Moderate to high levels of mycorrhizal colonisation were observed with all +AMF treatments and the proportion of colonised root was closely correlated to density of colonisation (Table 2.). Some low-level colonisation was observed following the addition of autoclaved AMF 2 & 3 (AMF 2-, AMF 3-) probably due to contamination, or from a few spores that survived the process of autoclaving. Both Symbio treatments produced a high level of colonisation – leading to doubts as to whether the Symbio MycoForce Potting Mix minus AMF propagules supplied for study was free of propagules. When the level and density of colonisation were compared within live and autoclaved treatments significant growth was observed for AMF 1+, AMF 2+, Symbio+ and Vaminoc+ compared to their respective minus AMF treatments at both levels of nutrients. The level of CRF had no significant overall effect on the level or density of colonisation. However, Symbio+ produced the greatest root colonisation with low CRF incorporation, whereas at the high CRF rate no mycorrhizas were observed, suggesting some form of suppression by the higher salt level.

Table 2. AMF level (AMF density)[1]

	AMF -		**AMF +**		**No AMF**	
	1.1 kg m^{-3}	***4.5 kg m^{-3}***	***1.1 kg m^{-3}***	***4.5 kg m^{-3}***	***1.1 kg m^{-3}***	***4.5 kg m^{-3}***
AMF 1	-	-	3.3 (3.3)	2.7 (2.7)		
AMF 2	0.7 (1.0)	0.7 (1.0)	2.3 (3.3)	3.0 (3.3)		
AMF 3	0.7 (0.7)	-	0.7 (0.7)	1.0 (1.3)		
PHC	-	-	1.0 (1.0)	1.0 (1.3)		
Symbio	1.3 (1.3)	3.3 (3.3)	4.0 (4.0)	-		
Vaminoc	-	-	2.3 (3.0)	1.3 (1.7)		
Control					-	-

[1] AMF level = the proportion of root length colonised by mycorrhiza: AMF density = the proportion of root cells containing mycorrhizal structures.

Growth responses

Overall, the amount of CRF incorporated had a significant effect on growth, with the high rate (4.5 kg m^{-3}) producing 25% larger plants than the low rate (1.1 kg m^{-3}). At the low CRF rate significant growth differences were observed between AMF 1+ and AMF 1- and No AMF control and Symbio- (Figure 1a). At the high CRF rate the application of 'active' treatments, namely PHC+ and AMF 1+, reduced growth compared to No AMF but the addition of autoclaved AMF 3- also significantly reduced growth (Figure 1b). Symbio- produced the greatest growth: significantly more than all other treatments.

Figure 1a. *Chamaecyparis lawsoniana* 'Columnaris'
Plant dry weight. 1.1 kg m^{-3} CRF

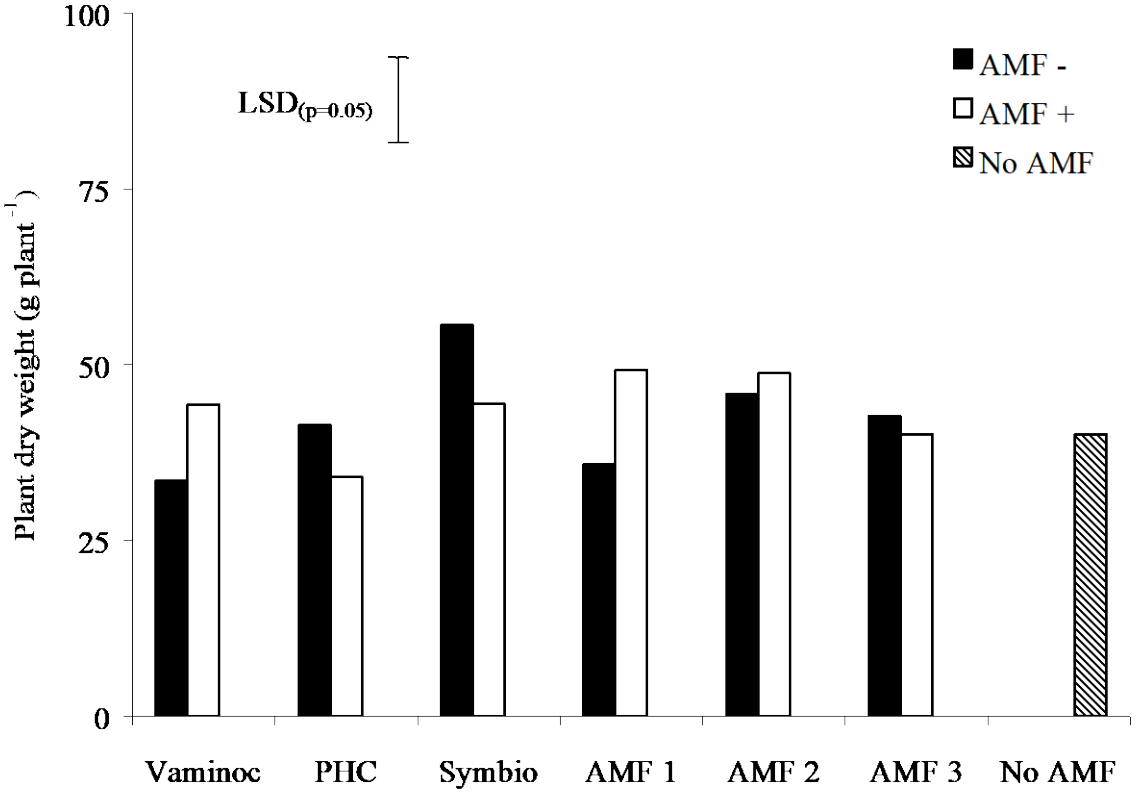
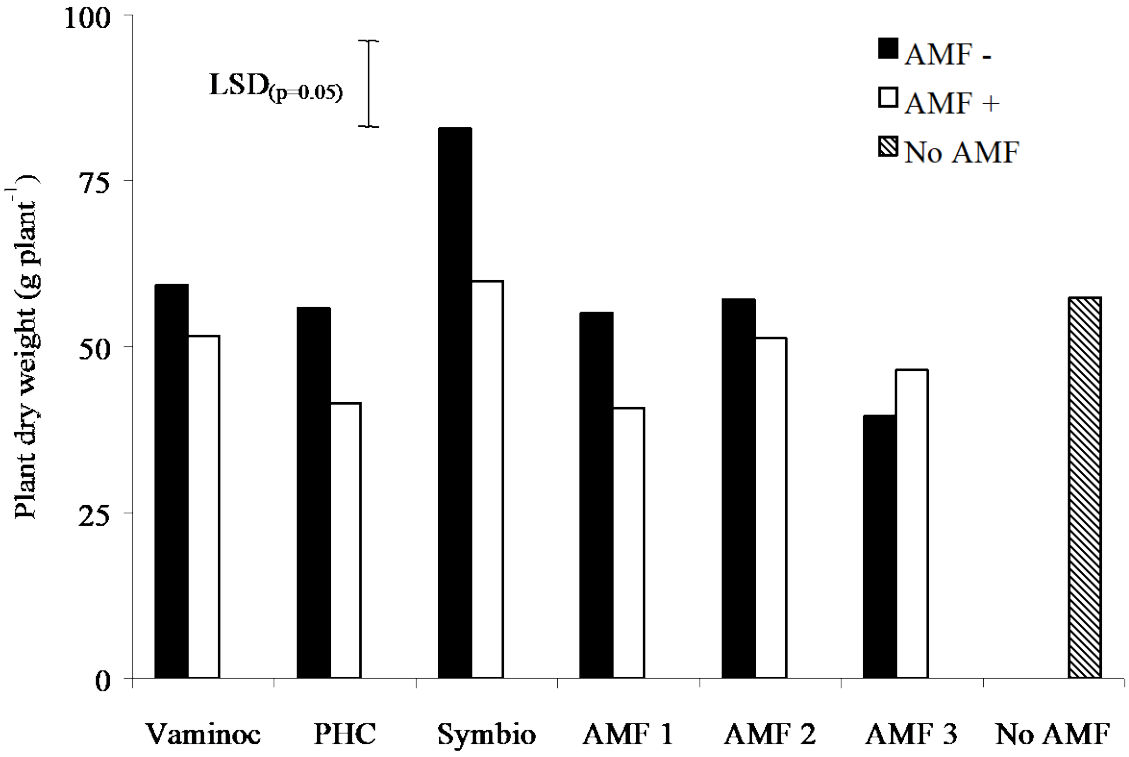


Figure 1b. *Chamaecyparis lawsoniana* 'Columnaris'
Plant dry weight. 4.5kg m^{-3} CRF



Nutrient responses

The only significant differences observed in foliage nutrient concentration were in response to the level of CRF incorporated in the growing medium. No significant difference was associated with presence of live propagules. Additionally, no correlation was observed between level of colonisation and foliage nutrient content for any nutrient.

Choisya ternata

Colonisation by AMF

Moderate AMF colonisation was observed following addition of the live treatments PHC+, Vaminoc+, AMF 1+and AMF 3+. Some contamination was apparent in the autoclaved Vaminoc- and AMF 1- treatments and the No AMF control. In the case of AMF 1, colonisation was greater in the autoclaved treatment compared to the live treatment. In contrast to *Chamaecyparis* no colonisation was observed with any Symbio treatment, and the level of nutrients had no effect on the extent of colonisation

Table 3. AMF level (AMF density)[1]

	AMF -		**AMF +**		**No AMF**	
	1.1 kg m^{-3}	***4.5 kg m^{-3}***	***1.1 kg m^{-3}***	***4.5 kg m^{-3}***	***1.1 kg m^{-3}***	***4.5 kg m^{-3}***
AMF 1	1.7 (1.3)	-	0.7 (0.7)	0.7 (0.7)		
AMF 2	-	-	-	-		
AMF 3	-	-	1.0 (1.3)	1.0 (0.7)		
PHC	-	-	1.0 (0.7)	1.0 (0.7		
Symbio	-	-	-	-		
Vaminoc	1.0 (0.3)	-	1.7 (2.0)	1.0 (1.0)		
Control					-	1.3 (1.3)

Growth responses

In general dry weight and size were closely correlated (as would be expected). However foliage colour was lightest in larger plants as a result of the darker foliage in the smaller, nutrient deficient plants. A phenomenon observed previously in HNS 43d. Overall, the incorporation of the high rate of nutrients (4.5 kg m^{-3}) produced plants twice as large as the low rate (1.1 kg m^{-3}).

[1] AMF level = the proportion of root length colonised by mycorrhiza: AMF density = the proportion of root cells containing mycorrhizal structures.

The most striking result was that of the addition of AMF 2, especially at the low CRF rate. Both the live and autoclaved treatments produced a marked increase in growth – approximately twice as much growth as the No AMF control (Figure 2a). The effect at the high rate of CRF was less dramatic and only in the autoclaved (AMF 2-) treatment (Figure 2b). However, study of the plant roots showed no colonisation at all. All components of the growing medium were common to other treatments (i.e. peat and CRF mix) except the experimental AMF treatment. As such some other unstudied factor is responsible for this result which is not affected by autoclaving. Both AMF 3 treatments also produced plants significantly larger than No AMF control at the high CRF rate. In contrast to AMF 2, colonisation was observed on the AMF 3+ plants; again the growth increase was due to some other unstudied factor which is not affected by autoclaving.

Figure 2a. *Choisya ternata*
Plant dry weight. 1.1 kg m^{-3} CRF

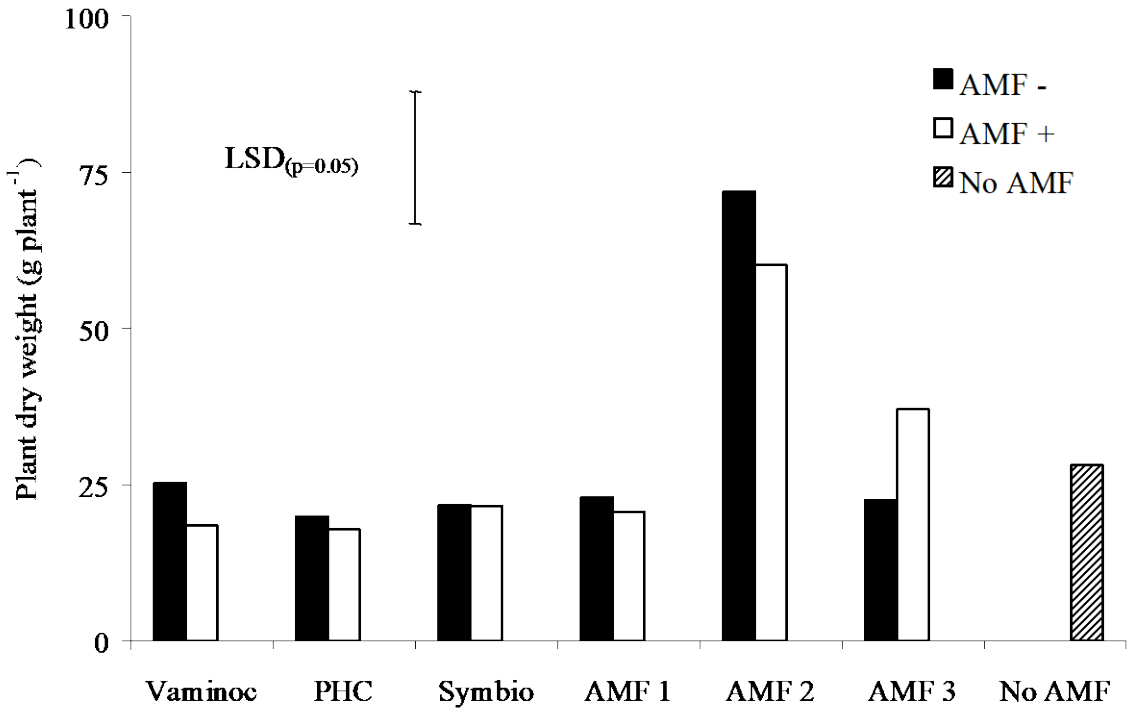
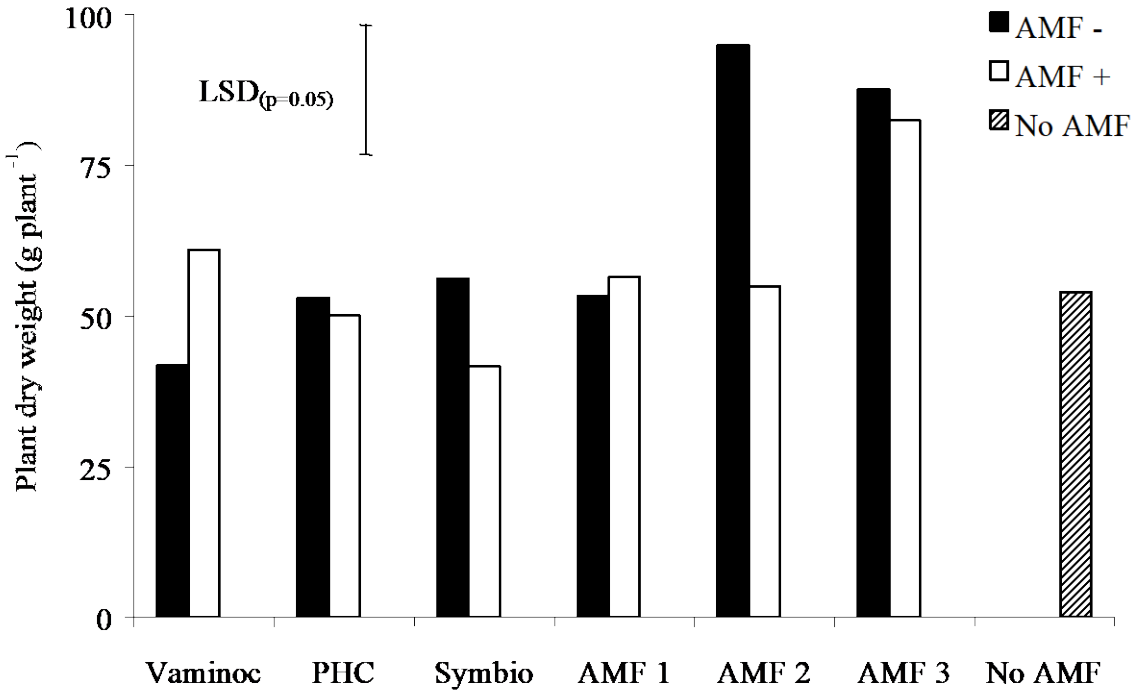


Figure 2b. *Choisya ternata*
Plant dry weight. 4.5kg m^{-3} CRF



Nutrient responses

The level of CRF incorporated in the growing medium was associated with significant difference in nutrient content of foliage and the weight of nutrient present in the plant. The AMF treatment showed few differences in the concentration of nutrients in the foliage (the exceptions being Ca and Mg) showing that any increased uptake was associated with increased size. However, no significant difference was associated with presence of live propagules. Additionally, no correlation was observed between level of colonisation and foliage nutrient content for any nutrient except Ca where the concentration of Ca in the foliage was correlated with the level of AMF colonisation.

***Lavandula angustifolia* 'Hidcote'**

Colonisation by AMF

Colonisation of roots was observed in all treatments except PHC- and Symbio- at the high nutrient rate (Table 4). No significant difference in colonisation was observed in response to CRF, or state of inocula (i.e live or autoclaved). Additionally, the No AMF treatment was colonised at a similar level to the other treatments. This suggests strongly that mycorrhizal propagules were introduced advertently or inadvertently during the commercial propagation of the plants, either through the addition of materials or from the standing area during weaning. It is unlikely that the growing medium used at Efford was the source of contamination as no large-scale contamination was present in the other two species. Propagules were never found in the medium, and no mycorrhizas became established in the bioassay control plants. Nevertheless, the extent of colonisation gives ideal material for studying small effects of colonisation on growth and nutrient acquisition. Assuming that there was a low-level contaminant in the treatments it is interesting to note that in a number of treatments, especially PHC- and Symbio-, root colonisation was inhibited at the high CRF rate.

Table 4. AMF level (AMF density)[1]

	AMF -		**AMF +**		**No AMF**	
	0.6 kg m^{-3}	***2.5 kg m^{-3}***	***0.6 kg m^{-3}***	***2.5 kg m^{-3}***	***0.6 kg m^{-3}***	***2.5 kg m^{-3}***
AMF 1	0.7 (0.7)	2.3 (2.3)	2.0 (2.0)	0.7 (0.7)		
AMF 2	0.3 (1.0)	0.7 (0.7)	3.0 (3.3)	2.7 (2.3)		
AMF 3	1.0 (1.3)	1.7 (2.3)	2.0 (2.0)	0.3 (1.0)		
PHC	0.7 (1.0)	-	1.0 (1.3)	2.0 (2.7)		
Symbio	3.7 (3.7)	-	1.7 (2.7)	1.0 (1.3)		
Vaminoc	1.7 (2.3)	2.7 (2.7)	3.0 (3.3)	1.0 (1.7)		
Control					2.0 (2.7)	2.3 (2.3)

[1] AMF level = the proportion of root length colonised by mycorrhiza: AMF density = the proportion of root cells containing mycorrhizal structures.

Growth responses

Plant dry weight and size were closely correlated. Plants grown with the high CRF rate (2.5 kg m^{-3}) were significantly larger than those grown with the low rate of CRF (0.6 kg m^{-3}) producing an average plant dry weight of 28.1 g and 18.4 g respectively. There was no significant difference among treatments at the low CRF rate (Figure 3a). However at the high CRF rate autoclaved PHC- significantly reduced growth compared to PHC+ (Figure 3b).

Figure 3a. *Lavandula angustifolia* 'Hidcote'
Plant dry weight. 0.6 kg m^{-3} CRF

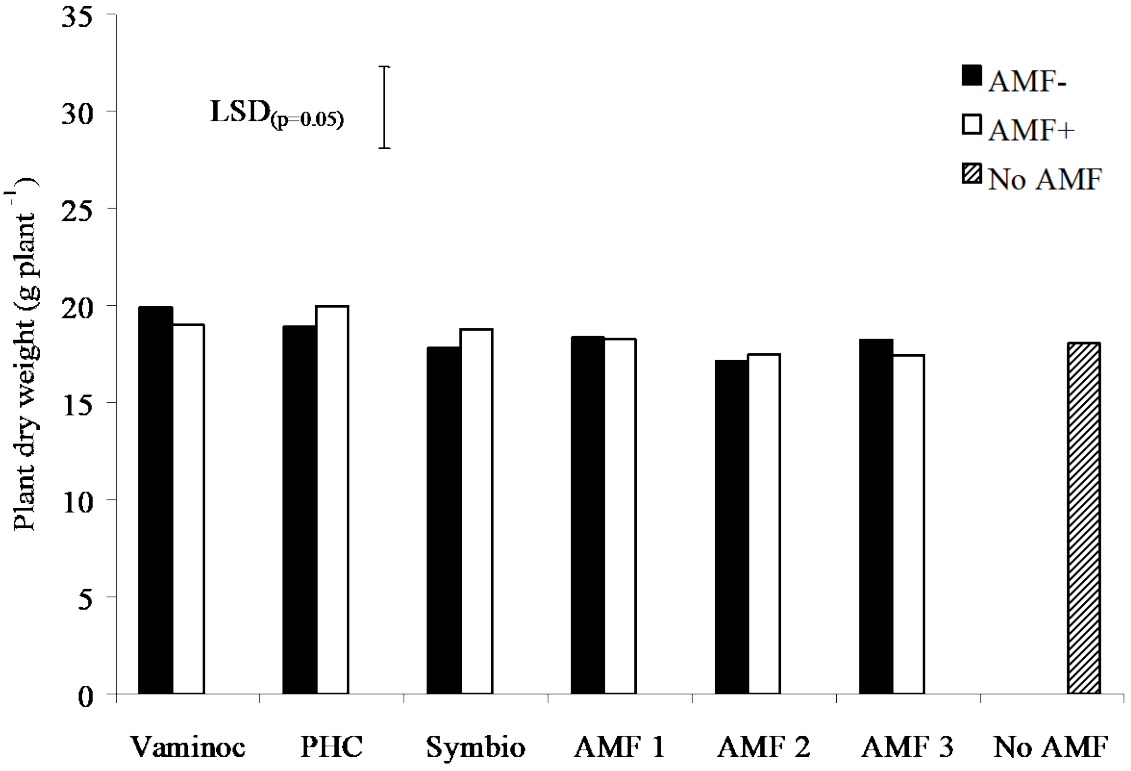
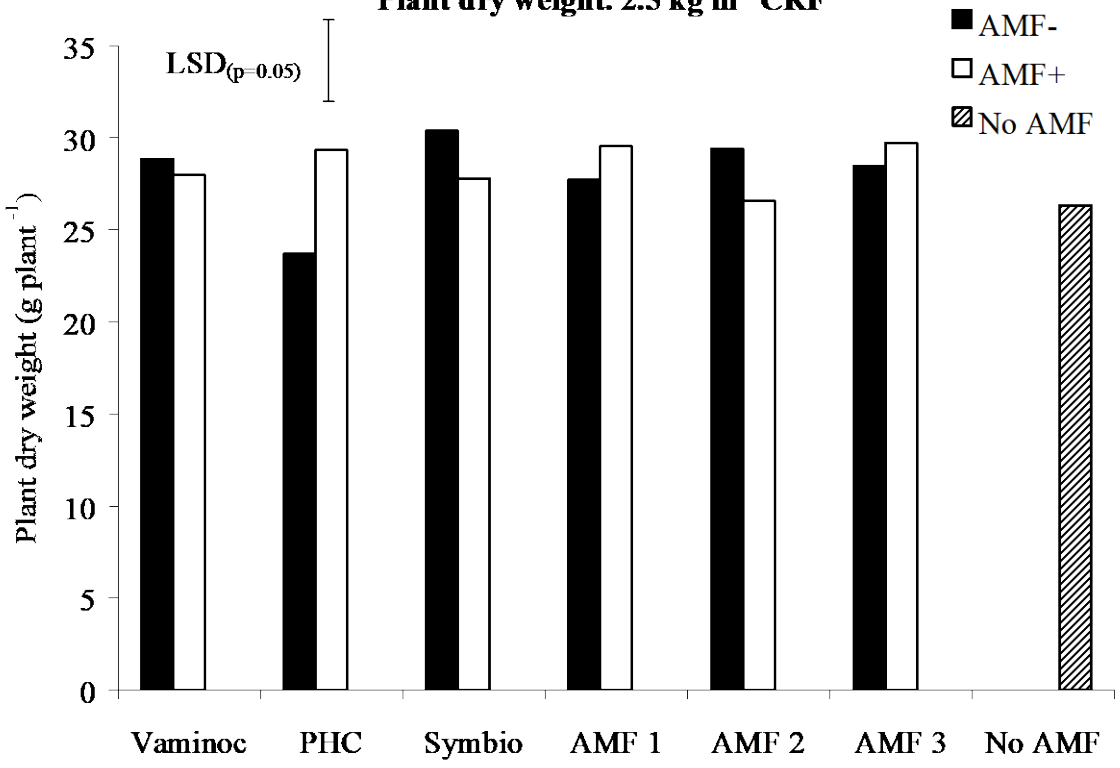


Figure 3b. *Lavandula angustifolia* 'Hidcote'
Plant dry weight. 2.5 kg m^{-3} CRF



Nutrient responses

CRF had an effect on all variables studied except foliar concentrations of nitrogen and potassium. Some significant differences were observed for phosphorous and calcium, with PHC+ having significantly more phosphorous, both as a concentration and absolute weight, than PHC- at both nutrient levels. In contrast, at the high CRF rate, Symbio- had a greater weight of calcium in the foliage than Symbio+. Overall, the extent of colonisation was correlated with a number of nutrients, but all of them negatively. The weight of magnesium and phosphorous accumulated by the plants was lower where AMF colonisation was high, and the concentration of phosphorous also decreased in association with increased AMF colonisation contrasting with the results from the PHC treatment.

Physiology Summary

Chamaecyparis showed good root colonisation following incorporation of AMF propagules in the growing mix. Growth responses in general were correlated with presence or absence of AMF. Interestingly, nutrient level influenced plant response to colonisation: at high nutrients plant growth was reduced; at low levels of nutrient growth was increased.

Of the treatments studied, colonisation of roots with AMF 1 enhanced growth at the low nutrient level but not so much as to substitute for a higher CRF rate. At the higher nutrient rate this effect was reversed. Symbio gave marked growth promotion with the 'no propagules' carrier mix at the high nutrient rate. However, root colonisation was very high suggesting that the Symbio – treatment contained viable propagules.

Choisya showed low root colonisation following incorporation of AMF propagules in the growing mix. In contrast to *Chamaecyparis* plant growth responses was not associated with presence or absence of AMF. The lack of colonisation explains the lack of response to treatment. However, a significant effect was observed with AMF 2 and AMF 3 where some unexplained factor was responsible for marked growth

Lavandula showed general colonisation across all treatments. The level of colonisation was generally greater in the treatments inoculated with viable propagules. It is likely that this contamination was introduced during the commercial propagation of the plugs bought in for the study. Plant growth did not respond to treatment or extent of colonisation of roots except for autoclaved PHC, which suppressed growth significantly at the high rate of CRF.

Nutrient uptake was influenced by AMF colonisation, with the overall relationship showing that to some extent high AMF colonisation was associated with low concentration and weight of Mg and P in the foliage. In contrast to the general trend, greater colonisation of roots of the PHC treatments was associated with increased concentration and weight of P in the foliage.

Pathology study (Year 2)

To determine the impact of AMF colonisation on plants' susceptibility to root rot

Materials and methods

Three AMF: Vaminoc, AMF 1 and AMF 2, were selected for this study based on their demonstrated abilities to colonise roots of *Chamaecyparis* plants. Plants were bought in from commercial propagators as rooted plugs and were potted into 3 litre pots. Inoculations with AMF were carried out at potting up on 8 June 2000 using the rates and conditions described for the crop physiology study. The inoculation treatments used for this pathology trial were Vaminoc+, Vaminoc-, AMF 1+, AMF 1-, AMF 2+, AMF 2- and uninoculated controls (see Table 1 for full descriptions). In addition, the impact of nutrition on the interaction between AMF, host plant and potential fungal root pathogens was considered using two application rates of controlled release fertiliser (Osmocote 12-14 Exact Standard 15+9+9+traces) of 4.5 and 1.1 kg m^{-3}. This gave a total of 14 treatments. Fifteen replicate plants per treatment were transferred from Efford sand beds to isolated HNS pathology beds (Pettitt, *et al.*, 1998). To avoid cross-infection between AMF treatments, each AMF treatment was restricted to a single isolated bed. Two repeat experiments were carried out using the same treatments. The first started in April 2001, the second in June. Both experiments were continued to a common termination date of end December 2001.

A severe *Phytophthora* root rot disease challenge was given to all plants using zoospore and mycelium preparations of four pathogenic *Chamaecyparis* root rot isolates of *Phytophthora* (*Phytophthora cryptogea* IMI 324217, A987; *P. cactorum* A547 and *P. cinnamomi* A558) following the schedule shown in Table 5. Progress of disease was regularly monitored by observation of the plants' shoots to determine an appropriate time to carry out thorough destructive assessments of disease.

By the end of the planned period for these experiments (end August 2001) the incidence of visible shoot symptoms was still only between 0 and 3% and it was therefore decided to extend the experimental period to the end of December 2001. This extension to the experiment allowed expression of the effects of the root rot pathogens on the autumnal root growth phase. To help with detection of pathogen propagules and bring on the expression of 'spring' shoot symptoms of root rot, plants were transferred to a glasshouse compartment maintained at > 8ºC at the start of December.

Detailed assessments of disease were carried out on all plants in both experiments over week 1, 2002. Shoot symptoms were assessed by a qualitative score of percentage of foliage affected by

'greying', wilting and/or browning. In addition, a record was made of any additional potential signs of disease, and of shoot heights in cm. Roots were assessed by a visual score of the percentage roots seen to be rotten when plants were emptied from their containers. Infection was assessed by (a) pot effluent water baits (Pettitt *et al.*, 1998) and by floating small samples of rotten root tissue in sterile pond water for 36 h prior to microscope observations of *Phytophthora* sporulation. A small sub-sample of roots were plated onto selective agar media to determine presence/absence of other potential root rot pathogens or secondary infections in affected roots.

Table 5. Pathogen inoculation schedules for experiments 1 and 2.

Date	Experiment 1	Experiment 2
06/04/01	Plants set out	-
12/04/01	1st inoculation: Zoospores 20 ml 10^4 spores ml^{-1} $plant^{-1}$. Isolates IMI 324217; A547 & A558	-
19/04/01	2nd inoculation: as above	-
22/05/01	3rd inoculation: as above	-
31/05/01	4th inoculation: as above	-
13/06/01	-	Plants set out
18/06/01	5th inoculation: Zoospores 20 ml 10^5 spores ml^{-1} $plant^{-1}$. Isolates A987 & A547;	1st inoculation: Zoospores 20 ml 10^5 spores ml^{-1} $plant^{-1}$. Isolates A987; A547 & A558
11/07/01	6th inoculation: as above	2nd inoculation: as above
20/08/01	-	3rd inoculation: as above
21/09/01	7th inoculation: Mycelial inoculum in sand oatmeal medium 10 g $plant^{-1}$. Isolate A987.	4th inoculation: Mycelial inoculum in sand oatmeal medium 10 g $plant^{-1}$. Isolate A987.
18/10/01	8th inoculation: as above	5th inoculation: as above
11/12/01	Plants moved to glasshouse	
7/01/02	Disease record started for both experiments	

Results

Although harvested on a single occasion, experiments 1 and 2 had been exposed to different pathogen inoculation schedules and infection conditions. The epidemics in each experiment would be expected to progress slightly differently and the results of each were considered separately.

The results of root float and baiting assays give an indication of the presence/absence of infection in individual root systems, but not more than a crude estimate of the severity of infections. The results of these two assays were combined in Figure 4 which shows that *Phytophthora* infections were positively identified in all treatments except for AMF 1+ inoculated plants. The severity of root browning was closely related to the incidence of infection, with consistently the greatest amount of browning in the two treatments where the highest incidence of infection occurred (Vaminoc- and AMF 2-, Figures 4 and 5). Negligible browning was recorded in the AMF 1+ treatment and also comparatively reduced browning was seen in the other two live AMF treatments Vaminoc+ and AMF 2+ (for illustration of severe root browning and healthy roots see Plate 4). In addition, the no AMF controls in experiment 1 unfortunately showed a comparatively low incidence of infection and consequently of root browning.

Using data from a parallel experiment on the amount of AMF colonisation of the roots of each treatment it was possible to assess the impact of AMF colonisation on root disease. Regression analysis (Figure 6) shows that the severity of root browning reduced with increasing level of AMF colonisation. This effect was more marked in experiment 2 than in experiment 1. The discrepancy between the two regression lines was largely due to differences in the amount of disease seen in control plants with no AMF colonisation in their roots.

Care is needed in interpreting this last result. It may be that low levels of infection and root browning in some of the control treatments in the first experiment were due to experimental error resulting from uneven drying rates following hand irrigation at the time of the initial pathogen inoculations. However, there may also be the possibility that at very low levels of AMF colonisation (perhaps representing a less specialised AMF/host relationship) in a root system might actually slightly increase the severity of root disease compared to no AMF controls. This question requires more detailed study, but for practical purposes Figure 6 demonstrates the importance of maximising AMF colonisation of host roots. Although root disease suppression by AMF infections is a widely reported phenomenon (Linderman, 1994), occurring in a broad range of host/AMF systems (Rosendahl, 1985; Newsham *et al.*, 1995; Cordier *et al.,* 1998) including *Chamaecyparis lawsoniana*/*Phytophthora* spp. (Bärtschi *et al.*, 1981), the presence of

Figure 4. Effect of AMF treatments on the incidence of Phytophthora root rot infections as detected by either root floats or pot-effluent bait assays.

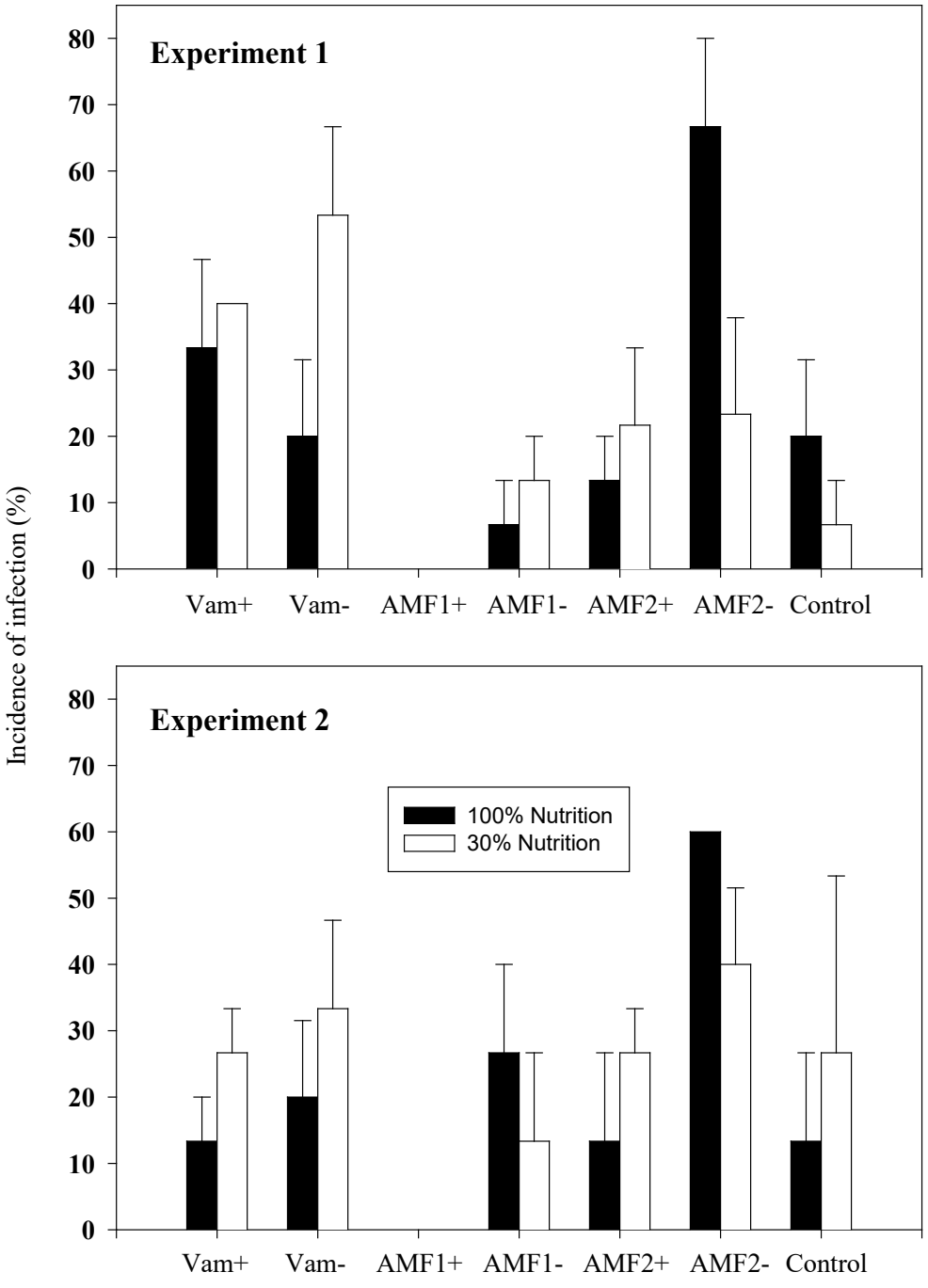


Figure 5. Effect of AMF treatments on the severity of *Phytophthora* root rot symptoms.

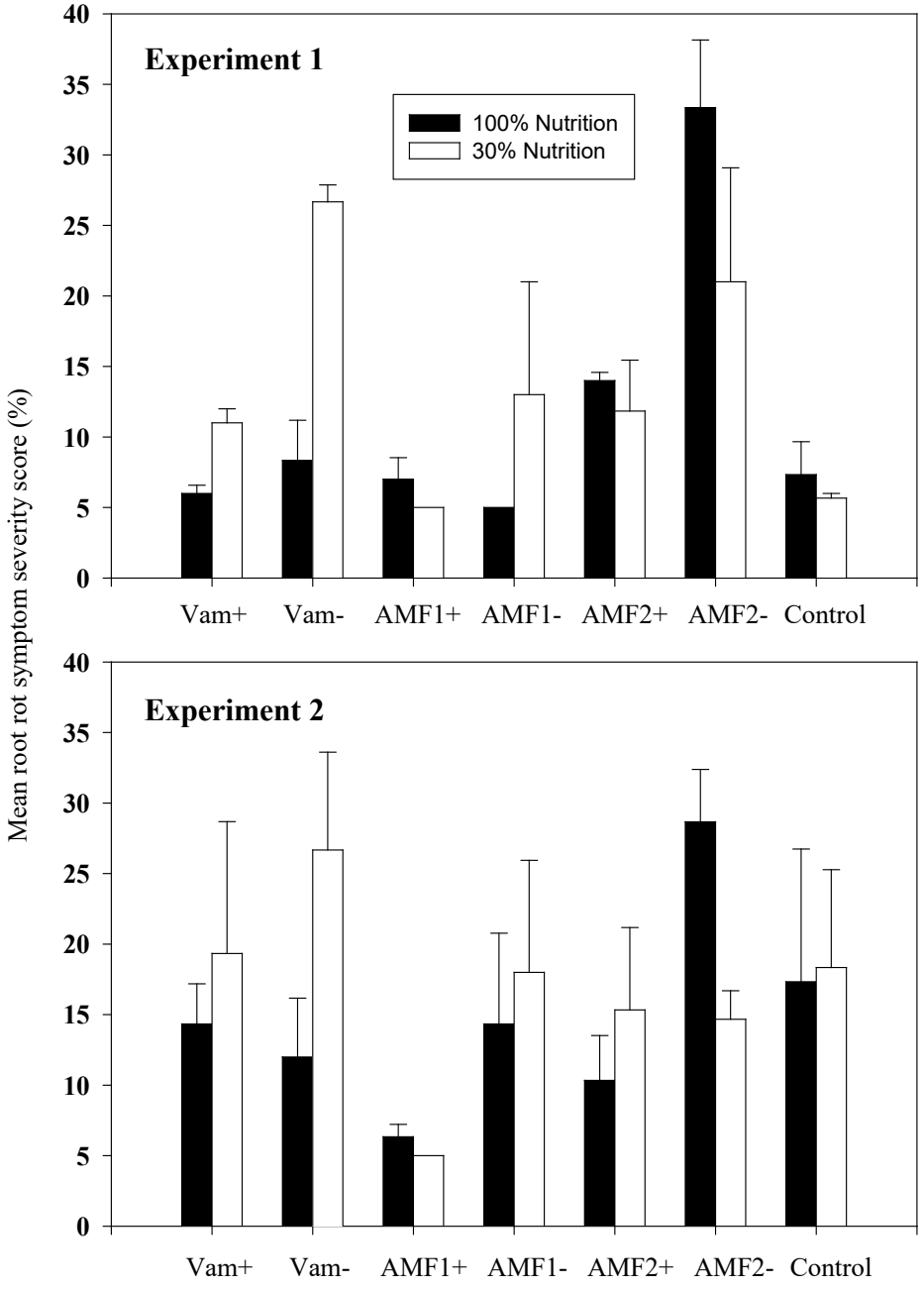
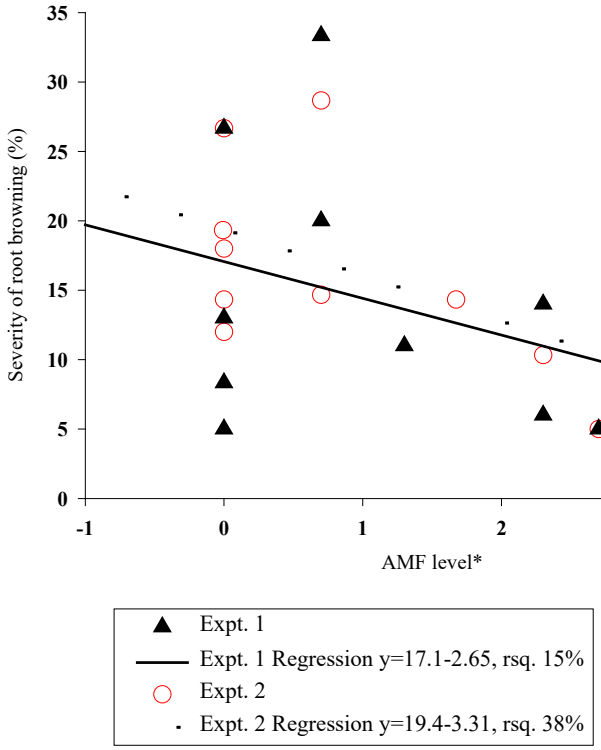


Figure 6. Assessment of the effect of the level of AMF colonisation in roots on the severity of root browning symptoms following inoculations with presence of AMF



* For details see Table 2.

Plate 4. Comparison between healthy (on right) and severely browned roots (on left).

AMF inoculum has not always resulted in disease suppression. Linderman (1994) indicates that in 17 out of 32 reports, AMF infection increased disease resistance, but in 12 no difference was observed and in 3 reports disease was actually increased. This variation in results could possibly be explained in terms of the degree of successful colonisation of the host species concerned.

The impact of the various treatments on plant height was not as strong as on root damage (Figure 7). However, the treatments with the lowest amounts of root browning did produce the tallest plants and there was a reasonably consistent although weak trend to decreasing height with increasing root damage (Figure 8). This result is not surprising considering the comparatively short duration of the experimental epidemics, and the effects of the root damage recorded would be expected to become more dramatic if plants had been kept under observation into the Spring of 2002.

More obvious was the incidence and severity of foliar symptoms (other than stunting!) of *Phytophthora* root rot. In *Chamaecyparis lawsoniana* 'Columnaris' early symptoms consist of an increase in the grey colouration of the foliage (Plate 5), sometimes accompanied by brownish undertones. As disease progresses the foliage feels dry to the touch and sometimes wilting occurs. Finally the foliage turns brown as the plant dies. Only a small proportion (<1%) of the plants had reached this last phase of disease and these were confined to the Vaminoc- and AMF 2- control treatments in experiment 1 (Figure 9).

In addition to these foliar symptoms a shoot 'tip-hooking' symptom was observed in both experiments 1 and 2 (Plate 6) and the incidence of this symptom was strongly associated with severe root browning. As with the other parameters of disease, no foliar symptoms of *Phytophthora* root rot were seen in any of the AMF 1+ treated plants (Figure 9). Other treatments all showed some foliar symptoms of disease, although symptom severity at the time of recording the experiments was still low.

As related above, there was some relation between shoot symptoms and root symptoms of disease and it would be reasonable to assume that the trends seen in the root symptoms would be re-enforced once a full flush of spring growth is under way. Some evidence of the first signs of a flush of spring shoot growth can be seen in the experiments as harvested but these are restricted to plants treated with the live AMF 1+.

Pathology Summary

These results show that AMF 1+ was the best treatment in terms of disease reduction. As stated above, this is likely to be due to the high level of successful root colonisation achieved with this AMF species. The limited evidence there is for the function of AMF in reducing root disease, underlines the importance of establishing a high degree of root colonisation by the AMF species

(Rosendahl, 1985; Linderman, 1994; Cordier *et al.,* 1998). Whether a high degree of colonisation of *C. lawsoniana* roots can be consistently achieved with inoculations using AMF 1 remains to be proven and further work would be required to determine how robust disease control using inoculations with this AMF species would be.

Figure 7. Effects of AMF treatments on plant heights following inoculation with a consortium of pathogenic *Phytophthora* root rot spp.

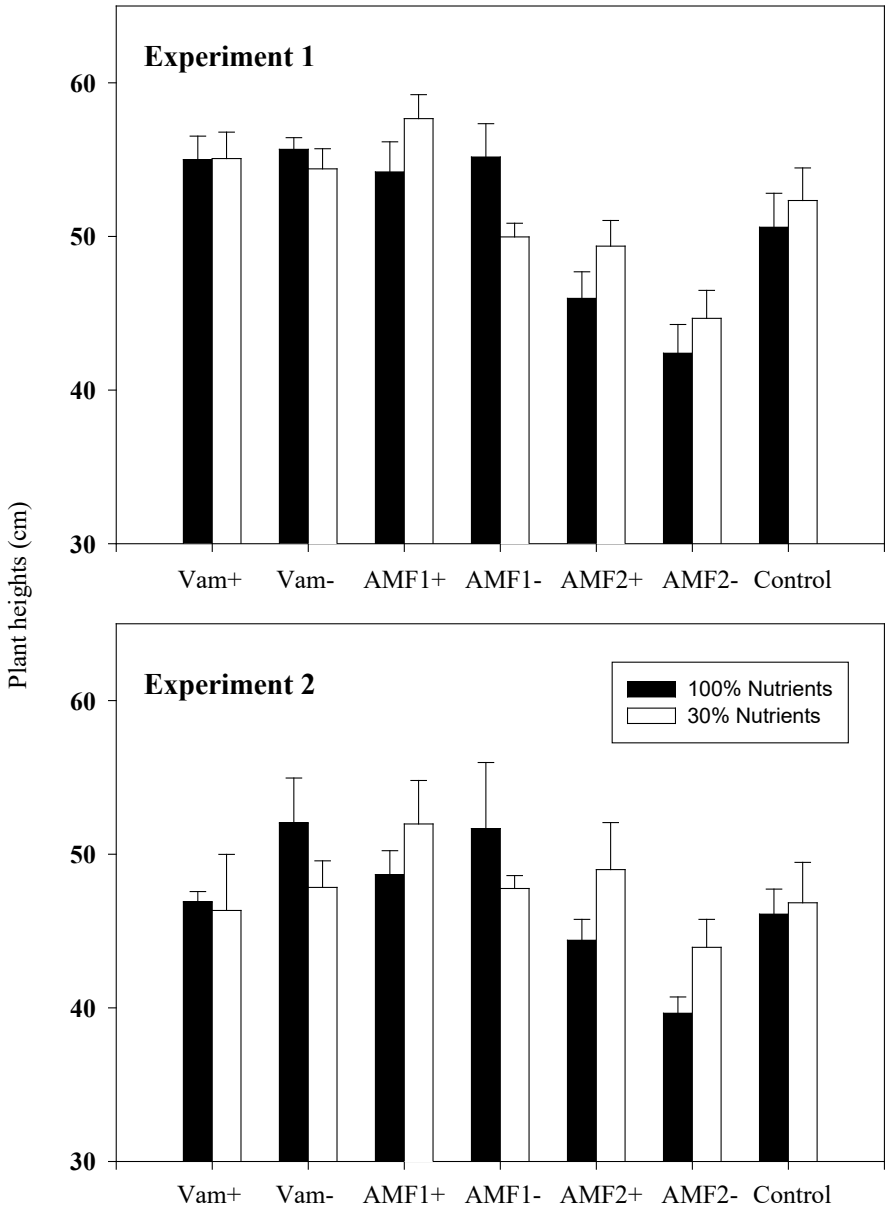


Figure 8. Figure showing weak correlation between root rot severity and plant height at the time of the final sampling (Jan. 2002).

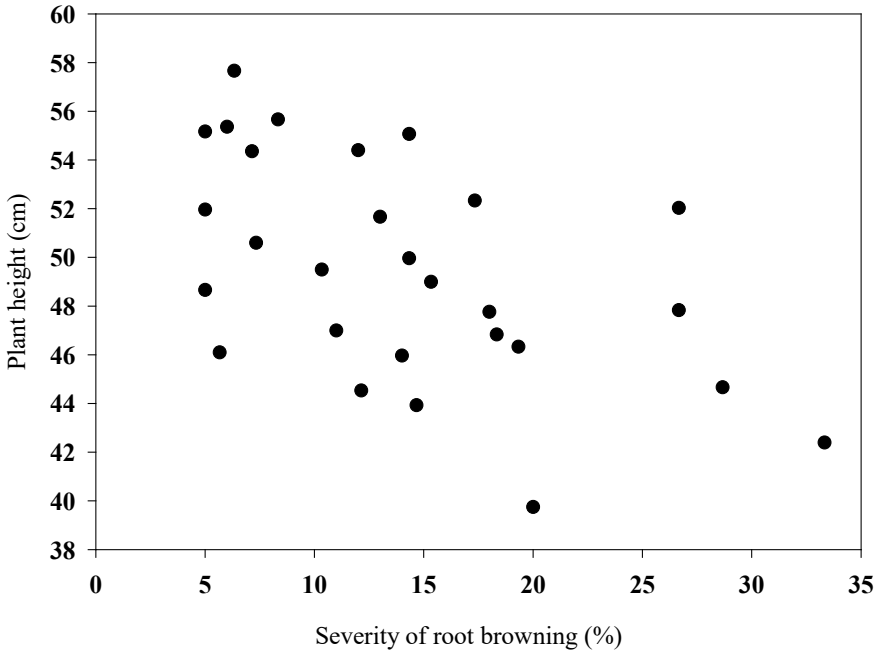


Figure 9. Effect of AMF treatments on the severity of foliar symptoms of *Phytophthora* root rot.

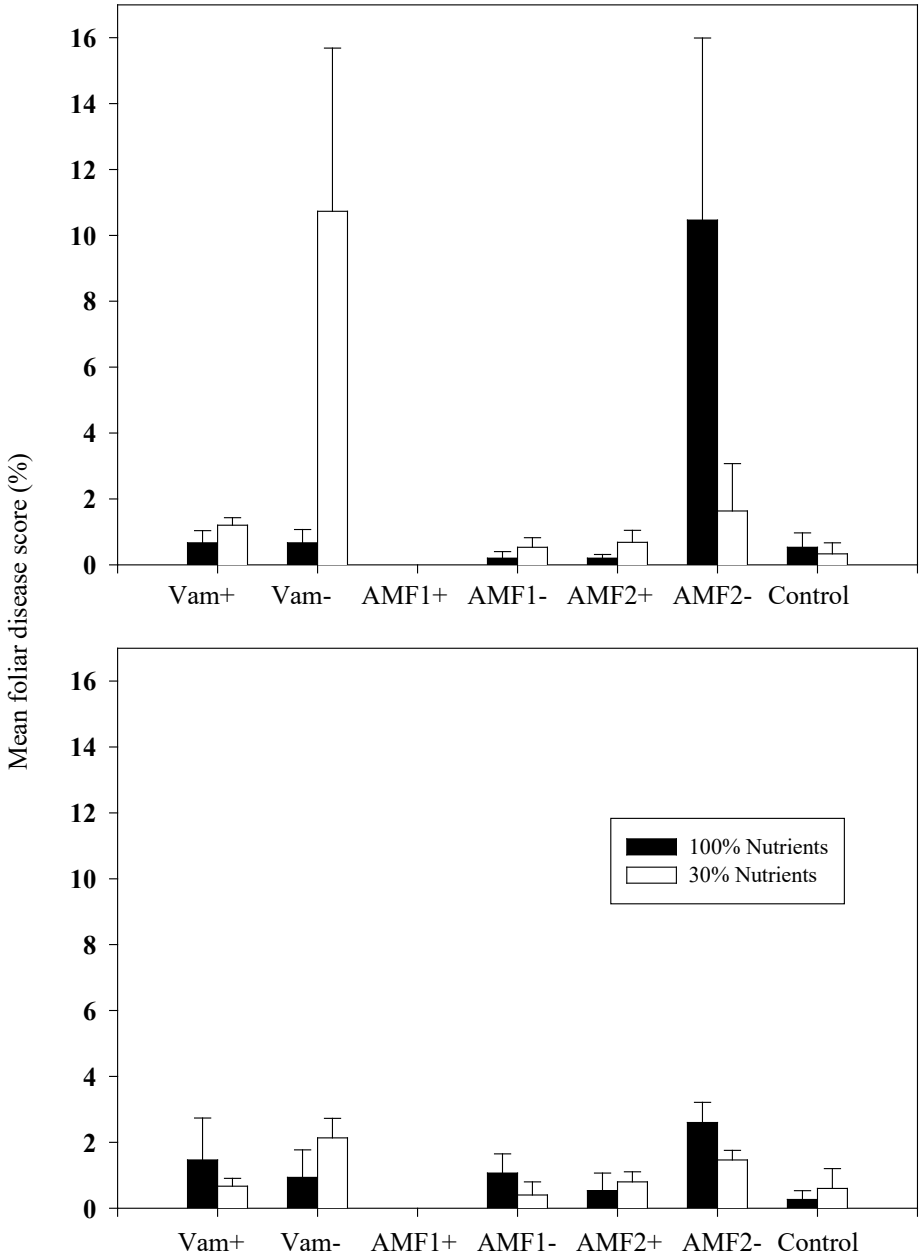


Plate 5. Comparison of foliar and root symptoms between healthy plant on right and a plant with severe root rot (>70%) and moderate (>10%) foliar symptoms of *Phytophthora* root rot.

Plate 6. Photograph showing typical example of the 'hooked shoot' symptom recorded in many plants showing severe root rot symptoms. This symptom is not a wilt, as the stem is quite rigid in this hooked habit.

General Discussion (Years 1 & 2)

This work was undertaken over two years to answer the following questions (each being an experimental objective):
1. Are the AMF in commercial products viable (and are their component fungi correctly identified)?
2. Do AMF improve rooting success if used in a peat-based propagation mix?
3. Can plants use less CRF if AMF are included in a peat-based growing mix?
4. If so, is this through increased efficiency?
5. Do AMF protect plants from root pathogens?

1. Are the AMF in commercial products viable (and correctly identified)

In bioassay 1 Chris Walker showed that although Vaminoc contained sufficient propagules at both bioassays, there were very low levels of propagules in Symbio MycoForce Potting Mix (Endo) and PHC Nursery Media Mix; too low for effective colonisation of roots during the propagation phase. The producers of PHC Nursery Media Mix and Symbio MycoForce Potting Mix (Endo) addressed problems of production and the products supplied for bioassay 2 were improved, and higher rates of incorporation recommended. Bioassay 2 showed that all products could colonise roots in optimum conditions (i.e. sand growing medium). By Bioassay 3 only Vaminoc colonised roots, again indicating variability in product viability with Symbio MycoForce Potting Mix (Endo) and PHC Nursery Media Mix.

Overall, the bioassays showed that AMF fungi were present, though not always in large quantities (judging from spore numbers). Spores are probably the main viable propagules in arbuscular mycorrhizal fungi, especially when their substrate has undergone dessication. The species present did not, for any inoculum, coincide completely with those said to be included. This should be a worry, as it is indicative of a need for much better quality control during production, and checking before marketing.

Even the species present did not all successfully sporulate. This could be because the spores were initially dead, or the fungi did not compete successfully with others in the mixture, or because the substrates or hosts were inappropriate for the particular fungi.

There were evident differences in the establishment of mycorrhizas related both to the host plant and the substrate used in the bioassays. These parameters are, however, somewhat confounded by the design of the trials (which were intended to demonstrate growth responses to viable commercial inocula). With the evidence from the bioassays and the pot trial, it is clear that there are interactions among substrates, plant species and fungal species or inoculum source. The presence of peat as a major component in the substrate is evidently deleterious to some AMF

species, though at least one commercial inoculum, produced in Canada, is grown in a peat-based substrate. Further, more specifically targeted experiments would allow these factors to be clarified.

2. Do AMF improve rooting success if used in a peat based propagation mixture?

This objective was pursued in year 1 and is discussed in depth in HNS 99 (annual report 1999-2000). Under the conditions studied, AMF colonisation of roots was rare and when observed was not associated with improvements in rooting success or plant growth. *Magnolia* roots were successfully colonised when Vaminoc was added to the rooting medium. With the other species studied only *Lavandula* exhibited any root colonisation, and even then at a low level and with Vaminoc only. It is interesting to note that *Magnolia* cuttings were in the rooting medium for 6 weeks longer than either *Chamaecyparis* or *Choisya*, and 10 weeks longer than *Lavandula*. It may be that effective colonisation will only occur over long periods of time (perhaps due to low numbers of viable propagules). If this is so, the potential applications for AMF inocula in commercial systems, where the pressure is for plants to spend less time in propagation, will be limited.

3. Can plants use less CRF if AMF are included in the growing mix?

The results from year 2 varied among host species and AMF treatments. Treatments were compared at approximately 30% and 100% of 'normal' CRF rate. With *Chamaecyparis* root colonisation was observed with all but one live treatment at both rates of CRF. At the highest rate colonisation was generally associated with a decrease in plant growth. This response has been reported in the scientific literature (e.g. Amijee, Stribley and Tinker, 1990) and is due to the 'cost' to the plant of supplying sugars to the fungus, but with no gain in nutrients as supply is adequate from the fertiliser. In contrast, at the low rate of CRF, root colonisation by AMF on average increased plant growth. One fungus isolate, AMF 1, identified as *Paraglomus occultum*, significantly increased growth from 40 to 50 g in dry matter production. However, this was still less than the weight of plants produced with the normal rate of CRF.

Choisya, whilst producing some interesting results, did not exhibit any increase or decrease in plant size as a result of AMF colonisation. Where differences occurred they were in plants uncolonised by AMF. In contrast, most treatments, regardless of presence of viable propagules, led to *Lavandula* root colonisation. The cause of this 'contamination' is unexplained. However, it allowed study of the association between extent of colonisation and growth response. There

were large differences between the two CRF rates but difference in plant size was not associated with AMF i.e. extent of colonisation with AMF had no effect on plant growth at either rate of CRF.

Overall, only *P. occultum* was able to improve growth at a reduced CRF rate and only with *Chamaecyparis*. Interestingly, addition of AMF at normal rates of CRF could actually reduce growth. There was no evidence to suggest that with the AMF studied either *Choisya* or *Lavandula* could maintain growth with a reduced rate of CRF through the addition of AMF.

4. If so, is this through increased nutrient use efficiency?

AMF colonisation was associated with no difference in the concentration or weight of nutrients in the foliage of *Chamaecyparis*. Hence, the increased growth associated with *P. occultum* at the low level of CRF was not due to increased efficiency of nutrient acquisition. Of note, AMF colonisation did influence nutrient uptake in both *Lavandula* and *Choisya*, but did not produce any increased growth.

5. Do AMF protect plants from root pathogens

There are many reports in the scientific literature of significant reductions in the amount of root disease seen in plants colonised by AMF (Linderman, 1994). In particular there are frequent reports of control of *Phytophthora* root rots (Davis & Menge, 1980; Cordier *et al.*, 1998) and even one previous report of control of *Phytophthora cinnamomi* in *Chamaecyparis* (Bärtschi *et al.*, 1981). The precise mechanisms for this reduction in disease are not clear and different AMF species may well operate in different ways. *Glomus fasciculatum* was shown by Meyer & Linderman (1986) to have a strong influence on the composition of the microbial populations in the rhizosphere, and indirectly to reduce the ability of *P. cinnamomi* to sporulate and infect new roots. Other workers have identified enhanced host resistance responses in AMF-colonised plants. For example Cordier *et al.* (1998) demonstrated the localised formation of cell wall appositions in AMF colonised roots, reinforced with callose which enclosed invading pathogen hyphae. These responses seem to be only weakly systemic, as demonstrated by Rosendahl (1985) in *Glomus fasciculatum* inoculated peas attacked by *Aphanomyces euteiches* (a pathogen from a group of fungi closely related to *Pythium* and *Phytophthora* spp.), in split root experiments. This explains why in the current project, the amount of disease reduction appeared to be inversely related to the level of AMF colonisation in the roots.

Of the three AMF treatments tested in the pathology trial, AMF1 (*Paraglomus occultum*), gave good results in terms of disease control, with no plants showing signs of disease after a prolonged

period of exposure to a severe *Phytophthora* root rot disease challenge. This result shows that *Paraglomus occultum* can be used for disease control. Unfortunately, it does not tell us whether disease protection was a direct result of the effects of *P. occultum* on disease or whether it was a result of the better level of root colonisation achieved in this project with *P. occultum*. Further detailed work would be required to determine this.

Paraglomus occultum was selected for this pathology study as a result of the failure of some of the commercial AMF preparations to colonise plants to an acceptable level. It was selected because it was originally isolated from an acidic peat substrate, and was therefore more likely to thrive in a typical HNS growing medium. The success of this selection demonstrates that there is likely to be still more rewards to be gained from assessing the ability of other similar accessions to suppress disease.

What factors limit AMF use?

Although we have shown that mycorrhizal fungi can be established on the roots of a range of HNS with commercial products or single isolates, their performance is variable and largely unpredictable. It is clear that the results from this work do not support the general application of AMF in commercial peat based propagation and growing of HNS. Significant positive responses have been achieved only with *P. occultum* (AMF 1). Nevertheless, an understanding of the potential causes of the lack of response with the commercial treatments, especially, may allow recommendations of changes to growing systems to gain from the evident benefits that AMF can provide in the natural environment. There are several factors that may influence the performance of arbuscular mycorrhizal fungi in a commercial production environment:

- Growing/rooting substrate
- Excess nutrients
- Use of fungicides
- Host species

These points are addressed individually below:

- ***Growing/rooting substrate***

It is evident that AMF colonisation of roots may be limited or even suppressed in a high nutrient environment or where incorporated into some of the 'younger', less mature blonde peats (Azcon-Aguilar & Barea, 1997). Where possible these factors were excluded from this experiment and for these reasons the bioassays were undertaken using sand culture with low levels of added nutrient. Nevertheless, this experiment sought to study the benefits of AMF in a *commercial growing system*, and to comply with this important experimental aim, a peat growing medium

was used. The Irish peat used was more mature than the Baltic peats also available in the UK, and it was hoped that this would minimise any possible 'suppressive' effect of the peat (HDC project PC 157; MAFF project HH1751SX). PHC Nursery Media Mix and Symbio Mycoforce Potting Mix (Endo) include both beneficial bacteria and fungi in their products, with claims that their presence enhances mycorrhizal colonisation.

In year 2, Bioassay 3 and 4 both showed that when inocula were introduced into a peat substrate colonisation of host roots fell markedly compared to a sand substrate, and Bioassay 4 confirmed that as the proportion of peat increased in the bioassay substrate successful AMF colonisation decreased. This suggests that the commercial products studied may not be ideally suited to a peat substrate. Another point to consider is the role of pH. Most of the fungi used in commercial mixes are thought to be isolated from neutral to slightly alkaline soils. The propagation mix was unlimed (~pH 4.5) and growing mix was limed to pH 5.5. This may be low for AMF colonisation with most of the fungi listed in the products. However, the success of *P. occultum,* having been isolated from a peat-based amateur growing substrate, demonstrates that there may be AMF suited to a peat growing substrate.

- ***Excess nutrients***

Whereas no nutrients were applied to the plants or rooting media during the propagation study CRF was added to the potting mix used in the growing-on phase. It was observed following a growing year that root colonisation was not limited at the higher nutrient levels studied, and with *Chamaecyparis* significant growth associated with AMF colonisation was only observed with Symbio MycoForce Potting Mix (Endo) at the high nutrient level. Nevertheless, it may be that nutrient levels best suited to colonisation and function were lower than studied at the 30% CRF rate. Work on-going (HNS 43f) studying optimal rates of fertiliser for a range of HNS has shown that *Choisya*, *Chamaecyparis* and *Lavandula* respond to CRF application up to 6, 4 and 4kg m^{-3}, respectively. Below these rates plant size decreases with reduced CRF. If CRF rates need to be less than the low rates studied here (1.1, 1.1 and 0.6 kg m^{-3} for *Choisya, Chamaecyparis* and *Lavandula*, respectively) for AMF to function, it is unlikely that final growth with AMF would be comparable to that with normal CRF rates and no AMF. For the grower, this is an important finding, since it is unlikely that loss of growth is an acceptable price to pay for the use of AMF!

- ***Use of fungicides***

Fungicides are routinely used in propagation to prevent potentially large losses, especially from damping off due to *Botrytis* species. The companies producing commercial preparations of AMF have carried out screening programmes for their products and provided information that guided our choice of chemicals. Non-systemic fungicides were used, and these were applied sparingly

to the foliage, not as a drench. None of these factors should prevent AMF colonisation, and the colonisation of *Magnolia* roots by Vaminoc confirms that the fungi were able to colonise in the conditions at Efford. No fungicides were used in the container crop, although Sus-con Green was incorporated into the growing media to prevent root grazing by vine-weevil (and potential contamination). It is possible (perhaps even likely) that this insecticide has deleterious effects on mycorrhizas, but it was impossible to pursue this line of enquiry in the present study.

- ***Host species***

During propagation, *Magnolia* roots were successfully colonised when Vaminoc was added to the rooting medium. With the other species studied only *Lavandula* exhibited any root colonisation, and even then at a low level and with Vaminoc only. Neither *Choisya* nor *Chamaecyparis* were colonised with any treatment. In contrast, with plugs inoculated at potting on, all three species studied (*Lavandula*, *Choisya* and *Chamaecyparis*) exhibited colonisation to some extent with some – and with *Lavandula*, most - of the AMF treatments. It can therefore be stated with confidence that all the species studied are able to form associations with AMF, and any failure to do so during propagation must have been due to other factors. However, it is becoming increasingly clear that, whilst not host-specific, there is a distinct host-fungus preference exhibited in mycorrhiza symbioses (Bever et al 1996).

Effect of biological growth promoters

The carrier material and added biological components of the treatments clearly influenced growth in a number of treatments. Two products contained growth promoters: Symbio MycoForce Potting Mix (Endo) and PHC Nursery Media Mix (the contents are listed in Appendix 1). With the **propagation** experiment, no consistent colonisation was observed with these two products and hence no growth response could be attributed to AMF. Nevertheless, large growth differences due to other components of the products were apparent for treatments. With PHC Nursery Media Mix only one variable (rooting success – *Choisya*) differed significantly between the product that was autoclaved (steam killed) and the product that was added under manufacturer's instructions (live). This shows there was no consistent effect derived from any living material included in the product. However, BioPak (containing beneficial bacteria and fungi) was watered on to the rooting medium of all host species and the actions of these organisms may have 'swamped' any differences.

Symbio MycoForce Potting Mix (Endo) produced an irregular pattern of response, some variables were best with the live AMF containing product and others with the same product minus the AMF propagules. The reasons for this irregularity cannot be explained from this study. It was interesting that significant deaths of *Chamaecyparis* cuttings occurred when

Symbio Mycoforce Potting Mix (Endo) was used. It seems possible that a pathogen, or pathogens, was introduced with the experimental treatments.

The role of carrier material was highlighted with *Lavandula*, where no plants rooted successfully if no AMF or carrier material was incorporated. The incorporation of either Vaminoc treatment resulted in a rooting success of 80-90%. No growth promoters were included with the Vaminoc product. Analysis of intrinsic fertiliser in the carrier material (from the growing media for the mother plants on which the AMF hyphae and spores are grown) showed that only minimal levels of nutrients were included in the product. The more probable explanation was that the change in the physical properties of the rooting medium, following the incorporation of a significant amount of clay carrier material, was beneficial to rooting.

The **growing-on** study produced colonised roots with PHC Nursery Media Mix (all three species) and Symbio MycoForce Potting Mix (Endo) (*Chamaecyparis* and *Lavandula*) making it difficult to separate growth responses due to either AMF or the other biological components.

The effect of AMF 2 and AMF 3 when incorporated into the growing medium of *Choisya* was also striking. However, study of the plant roots showed no colonisation at all. All components of the growing medium were common to other treatments (i.e. peat and CRF mix) except the experimental AMF treatment. As such some other unstudied factor or factors, not affected by autoclaving, must be responsible for this result.

Overall Conclusions (Year 1 & 2)

- The examination of commercial products showed that AMF fungi were present, though not always in large quantities. Spores are probably the main viable propagules in arbuscular mycorrhizal fungi, especially when their substrate has undergone desiccation. The species present did not, for any inoculum, coincide completely with those said to be included. This should be a cause for concern, as it may be indicative of a **need for much better quality control during AMF production**, and checking before marketing.

- Because AMF propagules in these products are grown with living plants there is the possibility that **pathogens could inadvertently be included**. For example, the bulbill of a possible mild pathogen (Papulospora sp.) was found in the Symbio MycoForce Potting Mix (Endo) inoculum, though there is no evidence that it was alive. Clearly it is important to eliminate any such possibility through high levels of quality control. The inclusion of plant pathogens in commercial products would not only be to the detriment of inoculated plants, but would be in breach of plant health regulations, particularly in those products imported from outside the European Union.

- There are indications from this work that **some factor in peat is limiting AMF colonisation** - whether this is due to organic content or pH could not be established from this work. Additionally the range of isolates studied was small and AMF isolates more suitable for use in peat growing media could certainly be isolated from suitable ecosystems. A few are already identified in scientific collections, but there is a great potential for the discovery, isolation and testing of new cultures that would be likely to have more potential as growth-promoters or in pathogen control in nursery stock production

- **AMF colonisation of roots was rare in the propagation experiment** and when observed was not associated with improvements in rooting success or plant growth. The cause or causes of this failure cannot be determined without further work. It is possible to speculate that low colonisation potential at recommended application rates coupled with the relatively short time that roots are available for colonisation and the biological properties of peat may contribute to this failure. If the former, then further investigations could be carried out to assess optimum dosage. If the latter, then the constraints of production systems will prevent any commercially viable use of these AMF formulations.

- In the **growing-on trial, root colonisation and plant responses were variable among treatments and host species**. Colonisation of roots with *Paraglomus occultum* (AMF 1) enhanced growth of *Chamaecyparis* at the low nutrient level but not so much as to substitute for a higher CRF rate. At the higher nutrient rate this effect was reversed. *Choisya* responded not to AMF colonisation, but to some other factor present in both PHC nursery

Media Mix and Symbio MycoForce Potting Mix (Endo). *Lavandula* showed no responses to either level of AMF colonisation or other factors in the commercial treatments.

- Nutrient uptake was influenced by AMF colonisation, with the overall relationship showing that to some extent **high AMF colonisation was associated with reductions in the concentration and weight of Mg and P in the foliage**.

- **Colonisation of *Chamaecyparis* with *Paraglomus occultum* appeared to reduce disease occurance and severity**. This may be due to the high level of successful root colonisation achieved with this AMF species rather than any 'special' quality. Whether a high degree of colonisation of *C. lawsoniana* roots can be consistently achieved with inoculations using *P. occultum* remains to be proven and further work is required.

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Appendices

Appendix 1. Products included in the trial

PHC Nursery Media Mix
Supplied by - Plant Health Care
Contact – Mr Jason Holohan
Contents - *Glomus clarum, G. etunicatum, G. intraradices, Entrophosphora colombiana*
Beneficial soil bacteria and fungi
Kelp extracts
Yucca extracts

Symbio MycoForce Potting Mix (Endo)
Supplied by - Symbio
Contact – Mr Martin Ward
Contents - *Glomus clarum*, *G. intraradices, G. mosseae, G. deserticola, G. monosporum, G. brasilianum, Gigaspora margarita*.
Beneficial soil bacteria and fungi
Kelp extracts
Yucca extracts
Humates

Vaminoc
Supplied by – MicroBio
Contact - Dr Ingrid Arias
Contents - *Glomus mosseae, G. fasciculatum*

PHC – BioPak
Supplied by - Plant Health Care
Contact – Mr Jason Holohan
Contents - Beneficial soil bacteria and fungi
Kelp extracts
Humic acid
Vitamins
Amino acids
Growth factors